Metamorphic changes in the haemocyte picture of the citrus butterfly, *Papilio demoleus* (L.) (Lepidoptera, Papilionidae)[1]

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Introduction

The haemolymph of insects can undergo quantitative changes to an extent virtually unknown for other tissues. In recent years increased attention has been paid to qualitative and quantitative studies of haemolymph in view of the fact that the internal environment in insects is regulated in its medium. Suggestions have been made for several haematological analyses of insect blood and their applications by Jones (1962) and Wittig (1963). The study presented here is an attempt to characterise the qualitative and quantitative aspects of blood picture of the citrus caterpillar, *Papilio demoleus*. Total haemocyte counts, differential haemocyte counts and blood volume determination have been made in the larval and early pupal stages to study the changes associated with metamorphosis.

Materials and Methods

The citrus caterpillars that are about to moult into third instar which can be recognised by the change in colour from brown to yellowish green were used in the study. Care was taken to keep the groups of test material homogenous by way of selecting 10-12 days old larvae based on colour changes and 1-2 days old pupae. For the differential haemocyte counts the caterpillar was heat-fixed at 52-56°C for a minute or two, and by puncturing the first abdominal proleg, a drop of blood was collected on a clean, grease-free slide. The blood smear preparation was stained with Giemsa stain and the various types of haemocytes were classified according to the recent classification given by Jones (1962) and Patton (1963).

For the total haemocyte counts, the haemolymph was collected in a Thoma White blood cell pipette to the 0.1 mark, diluted with 1.5 per cent acetified saline solution to prevent clotting, and made up to 11 mark. The blood cell counts were made by using double ruled Neubauer Haemocytometer. The number of haemocytes per mm^3 of blood was calculated by counting the cells of five 1 mm square areas in each of the two chambers by using the formula given by Jones (1962):

$$\frac{\text{Haemocytes in five mm}^2 \times \text{Dilution factor} \times \text{Depth factor}}{\text{Number of squares counted}}$$

The procedure for dye dilution technique

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described by Lee (1961) was adopted using Congo red for the blood volume determination. Fifty ml of Congo red equivalent to 0.125 mg of the dye was injected into the haemocoele through the first abdominal proleg and inserted upto the thorax region. After five minutes, 100 ml of blood was drawn and made up to 7 ml with stock solution of sodium chloride and its optical density determined using Systronics colorimeter at 480 mμ with a blue filter. In the case of 1-2 days old pupae, owing to the small quantity of the haemolymph, only 50 ml of blood was drawn, made up to 7 ml and optical density determined.

From the optical density values of the sample, the amount of the dye in the blood sample was determined by referring to a standard graph. From the amount of dye injected and the amount of dye in the blood sample, the blood volume was determined by the formula given by Lee (1961):

$V = (dg_1/g_2)$—a, where 'V' is the volume of blood, 'g_1' is the weight of the dye injected, 'g_2' is the weight of the dye in the sample, 'd' is the volume of the sample and 'a' is the volume of saline injected with the dye.

To this value is added 100 ml as 100 ml of blood was drawn from the test insect in the case of larvae and 50 ml in the case of pupae to serve as blanks. In the estimation of the blood volume, the wet weight of the animal is important, as the water imbibed by the insect constitutes to the dilution of the blood and a certain proportion to hydration of tissues. The wet weight was determined after removing adhering water from the insect which was wiped well with folds of filter paper. The blood volume was expressed as percentage of wet weight of the animal in mm^3.

Results and discussion

The blood of the larva and pupa of *Papilio demoleus* was pale yellowish green in colour and watery in consistency. It took about 35 minutes to clot when extracted from the heat fixed sample. The clotting of the blood was accompanied by a change in colour to dark green and ultimately black.

1. *Differential haemocyte counts*

There is much confusion in insect haematology since few authors have agreed on a common terminology. A critical discussion of the problem of blood cell classification was recently put forth by Jones (1962), and in the present paper the terminology suggested by him and Patton (1963) has been adopted. The cell types namely prohaemocytes or proleucocytes, plasmatocytes, spindle-shaped cells and vermiform cells could be distinguished in the haemolymph of *P. demoleus* larvae. The plasmatocytes constitute the major portion of the haemocytes counted, followed by prohaemocytes, spindle-shaped cells and vermiform cells.

The prohaemocyte has a large nucleus and very little cytoplasm whereas the plasmatocytes have well defined cytoplasm and small nucleus. The cytoplasm of this type of cell may be relatively clear, or in certain cells granules and vacuoles may be found. The spindle-shaped cells are also with small nucleus and well defined cytoplasm with vacuoles. In the case of vermiform type of cells, both the ends of the cell are twisted in a thread-like manner and have small nucleus with large cytoplasm. The spindle-shaped cells and vermiform type of cells now observed in *P. demoleus* larvae may be nothing but the plasmatocytes which at times may send out pseudopodia or get rounded up, or twisted at both the ends as suggested by Jones (1962) and Patton (1963). When viewed edgewise the plasmatocytes may become spindle-shaped.

2. *Total haemocyte counts*

It is seen from Table 1 that the total haem-

ocyte counts (THC) of heat-fixed larvae ranged from 17500 to 22750 cells/mm^3 with a mean of 21541 cells. In the case of pupa, the blood cells varied from 2250 to 3250 cells/mm^3 with a mean of 2812 cells. It is evident that there has been a 7.7 fold decrease in the number of haemocytes from the actively feeding larval stage to the inactive pupal stage.

That the total and differential haemocyte counts are diminished in the pupal stage has also been reported in *Anagasta kuhniella* (Zeller) (Arnold 1952), *Bombyx mori* (L.) (Nittono 1960), *Pectinophora gossypiella* (Saunders) (Clarke & Chandbourne 1960), *Prodenia eridania* (Cramer) (Yeager 1945; Rosenberger & Jones 1960), *Galleria mellonella* L. (Jones 1967a) and *Sarcophaga bullata* P. (Jones 1967b).

3. *Haemolymph volume*

The haemolymph volume expressed as percentage of the body weight of the larvae ranged from 29.48 to 37.20 with an average of 32.94 (Table 1). In the case of pupae the volume ranged from 15.90 to 19.87 per cent with a mean of 17.53. The volume has been decreased by 46.8 per cent in the pupa. This reduction is in accordance with the observations made by Lee (1961) in the case of locust *Schistocera gregaria* in which the volume of 22.0 per cent in nymph was reduced to 13.95 per cent in the newly formed adult. Similarly Jones (1967a) found that the haemolymph volume declined from 34 per cent in precocoon spinning larvae to 16.4 per cent in the newly formed pupa in *Galleria mellonella.* These observations indicate that fluctuations in blood volume are related to the stage of development of an insect.

The results presented above on THC and haemolymph volume of both larvae and pupae may be combined to indicate the changes in the haemocyte population within the whole insect. When the THC was multiplied with haemolymph volume the calculated haemocyte population could be arrived at. The estimated mean number of haemocytes in the whole larva was found to be 7,09,562 as against only 49,226 in the pupa (Table 1).

TABLE 1

HAEMOCYTE COUNTS, BLOOD VOLUME AND EXPECTED POPULATION OF HAEMOCYTES IN THE LARVAE AND PUPAE OF *Papilio demoleus* L.

Particulars	Larva	Pupa	% decrease in pupa	Levels of significance (%)	S.E.	CD.
1. Total Haemocyte counts (THC)	21541.30	2812.5	86.9	1	1381.0	4289.3
2. Blood volume (% on wet weight basis)	32.94	17.53	46.8	1	2.1	6.5
3. Expected Haemocytes population (THC x Blood volume)	7,09,562	49,226	93.0	1	19055	50184

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References

Arnold, J. W. (1952): The haemocytes of the Mediterranean flour moth, *Ephestia kuhniella* Zell. (Lepidoptera: Pyralidae). *Can. J. Zool. 30*:352-364.

Clarke, E. W. & Chandbourne, D. S. (1960): The haemocytes of non-diapause and diapause larvae and pupae of the pink bollworm. *Ann. Entomol. Soc. Am., 53*:682-685.

Jones, J. C. (1962): Current concepts concerning insect haemocytes. *Am. Zool. 2*:209-246.

——————— (1967a): Changes in the haemocyte picture of *Galleria mellonella* Linnaeus. *Biol. Bull., 132*:211-221.

——————— (1967b): Estimated changes within the haemocyte population during the last larval and early pupal stages of *Sarcophaga bullata* Parker. *J. Insect Physiol., 13*:645-646.

Lee, R. M. (1961): The variation of blood volume with age in the desert locust (*Schistocerca gregaria*). *J. Insect Physiol.* 6:36-51.

Nittono, Y. (1960): Studies on the blood cells in the silkworm, *Bombyx mori* L. *Bull. Sericult. Expt. Sta. (Tokyo), 16*:171-266.

Patton, R. L. (1963): The function of transport-blood and circulation. *In* "Introductory Insect Physiology", R. L. Patton (ed.) 46-66. W. B. Saunders Company, London.

Rosenberger, C. R. & Jones, J. C. (1960): Studies on total blood cell counts of the Southern army worm larvae, *Prodenia eridania* (Lepidoptera). *Ann. Entomol. Soc. Am., 53*:351-355.

Wittig, G. (1963): Techniques in Insect pathology. *In* "Insect pathology: An Advanced Treatise". E. A. Steinhaus (ed.) 2:591-636. Academic Press, New York.

Yeager, J. E. (1945): The blood picture of the Southern army worm (*Prodenia eridania*). *J. Agr. Res., 71*:1-40.